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GENETICAL AND PHYSIOLOGICAL STUDIES ON COLLYBIA VELUTIPES

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INAUGURAL DISSERTATION

BY

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Introduction.

The present paper is, in the main, a summary of the following earlier communications, to which references will be made by means of Arabic numerals:

1. Aschan, K., 1952: Studies on dediploidisation mycelia of the basidiomycete *Collybia velutipes*. —Svensk Bot. Tidskr., 46: 366–392. Uppsala.
2. —»—, 1954 a: Some facts concerning the incompatibility groups, the dicaryotization and the fruit body production in *Collybia velutipes*. — Svensk Bot. Tidskr., 48: 603–625. Uppsala.
3. —»—, 1954 b: The production of fruit bodies in *Collybia velutipes* I. Influence of different culture conditions. — Physiol. Plant., 7: 571–591. Lund.
4. Aschan-Åberg, K., 1956: Studies on a homothallic mycelium derived from two mutants of *Collybia velutipes*. — Svensk Bot. Tidskr., 50: 385–398. Uppsala.
5. —»—, 1958: The production of fruit bodies in *Collybia velutipes* II. Further studies on the influence of different culture conditions. — Physiol. Plant., 11: 312–328. Lund.
6. —»—, 1960 a: The production of fruit bodies in *Collybia velutipes* III. Influence of the quality of light. — Physiol. Plant., 13: 276–279. Lund.
7. —»—, 1960 b: Studies on mono- and di-caryotic mycelia of *Collybia velutipes*. — Physiol. Plant., 13: 280–297. Lund.

8. ASCHAN-ÅBERG, K., 1960c: Studies on dedicaryotization mycelia and of F_1 variation in *Collybia velutipes.* — Svensk Bot. Tidskr., 54: 311–328. Uppsala.

The investigation was originally planned as a continuation of the work of HARDER (1927), who showed that the clamp-forming basidiomycetes may constitute an excellent material for studies on cytoplasmic and nuclear inheritance and their interrelations. The agaric *Collybia velutipes* was chosen as an object primarily because of its production from dicaryotic mycelia of normally monocaryotic oidia. Plating out of such oidia was a convenient means of obtaining neohaplonts (FRIES & ASCHAN 1952) in addition to the more laborious method of microsurgical operations (*1*). Schemes of the life cycle of *Collybia velutipes* and of clamp formation are given in *1*: Figs. 1 and 2. In most respects the fungus behaves similarly to *Schizophyllum commune*, another basidiomycete of the family Agaricaceae which during the last decade has been the object of detailed genetic studies (cf. PAPAZIAN 1958, RAPER & MILES 1958, JÜRGENS 1958). It deviates, however, from *Schizophyllum* in its capacity to form oidia.

It soon became apparent that the neohaplont studies should be extended by studies on segregation of various factors in the F_1 progeny. To this end the mating relations and the conditions leading to fruit body production had to be studied (*2*, *3*, *5*, *6*, *7*). It is true that most dicaryotic strains of *Collybia velutipes* easily produce fruit bodies in pure cultures (cf. PLUNKETT 1953) but in some cases (*4*, *8* p. 315) all efforts to obtain them have been in vain.

Material.

The precise taxonomic position of *Collybia velutipes* (CURT. ex FR.) QUÉL. is open to doubt (cf. *4* p. 394). It is a wood-rotting agaric appearing during the colder part of the year on slightly decayed stumps or trunks of various deciduous trees (STEWART 1918, LANGE1935–40).

Fruit bodies of abnormal structure are known to occur (STEWART 1918) and in rare cases dwarfish forms have been observed on coniferous wood (LANGE 1935–40). Such deviating types would have been valuable for genetic studies, but were not included in material from 63 different localities in different parts of Scandinavia obtained during the winter 1951–52 through the kind help of a large number of botanists. The fruit bodies were of a rather uniform appearance,

and no distinct basidiospore types could be discerned in spite of a fairly wide range of variation in respect to size and form. Also the mycelia obtained from basidiospores of 30 different fruit bodies (about 15 from each of them) were of a similar growth type though differing in colour (from white to yellowish and yellow-brown). The origin of the wildtypes used for further studies as well as the mutant strains obtained from them mainly by N-mustard treatment are described in paper (*1*) and by ASCHAN & NORKRANS (1953).

Most of the strains have been rather constant during several years of culture, but a gradual "degeneration" has occurred in some cases (*7*, *8*).

This material has, in addition to the studies reported in (*1–8*), been used for studies on cellulolytic enzymes (ASCHAN & NORKRANS 1953, NORKRANS & ASCHAN 1953, NORKRANS & RÅNBY 1956, NORKRANS 1957) and for studies concerning the effect of fructose-1,6-diphosphate on growth (NILSSON 1956).

Germination of basidiospores (*2*, *8*).

The question of the viability of the basidiospores became topical at first in connection with studies on the incompatibility groups (*2*), which seemed to show that mycelia obtained from basidiospores of certain fruit bodies mated according to a dipolar scheme. The germination frequency was found to be rather low, but a hypothesis concerning linkage between one of the missing incompatibility factors (for example b_1) and a lethality factor could not be verified by such studies. The germination frequency was in fact as low (or perhaps lower) for basidiospores from fruit bodies giving rise to F_1-mycelia mating according to an irregular pattern with indications of tetrapolarity (*2*, Fig. 1). As mentioned below, it was later found that the dipolar behaviour was not genuine, but depended on the occurrence of illegitimate matings.

It is a disadvantage to use spores of low viability in connection with segregation analyses, and it therefore became pertinent to make further studies on the germination frequency of basidiospores of different origin (*8*). This frequency varied strongly for spores obtained from different fruit bodies generated by identical mycelia, which might be connected with variations in spore age or of the environmental conditions prevailing during spore formation. However, there was a clear tendency to lower germination frequency when

one or both of the parental monocaryotic strains were mutants than when they both were wildtypes. Spores from two haploid fruit bodies of a wildtype strain showed a rather low germination frequency.

Distribution of oidial neohaplont types (*1*, *8*).

It was reported by BRODIE (1936) that oidial neohaplonts produced by dicaryotic *Collybia velutipes* strains were almost equally distributed between the original monocaryotic types used for the synthesis of the dicaryotic mycelium. In contradistinction, the present author has usually found very uneven ratios between the oidial neohaplont types (*1*, *8*). Sometimes one of the expected types totally failed to occur and could not even be isolated by use of a selective medium (tested for one biochemically characterized type). No clear evidence for an effect of the age of the mycelium producing the oidia upon the neohaplont ratio could be found. On the other hand, there is some correlation between the frequency of a certain neohaplont type and the tendency of the corresponding monocaryotic mycelium to produce oidia. This may be the natural outcome of the production of oidia from monocaryotic hyphae arising on the dicaryotic mycelium by spontaneous dedicaryotization. A type which is easily obtained from some dicaryotic combinations may, however, be lacking among the oidial neohaplonts produced by another combination, which indicates an influence of the dicaryotic mycelium, perhaps via cytoplasmic factors, upon the hyphae producing the oidia.

Incompatibility groups (*2*, *8*).

The first experiments (*2*) concerning the mating behaviour of basidiospore mycelia resulted in pairing patterns of the dipolar type or in an irregular pattern. As *Collybia velutipes* had earlier been shown to be a tetrapolar species (for references, see *2*; also TAKEMARU 1954) this was rather unexpected and prompted more detailed studies (*2*). These soon showed that in many cases the clamp formation was restricted to the contact zone between the mycelia, while in other cases a complete dicaryotization on both sides of this zone took place. If the incomplete ("illegitimate") pairings were discarded a tetrapolar scheme resulted. The incomplete dicaryotization occurred in the border zone between mycelia "heterozygotic" in respect to factor "a" but "homozygotic" in respect to "b" (as in $a_1b_1 + a_2b_1$ and $a_1b_2 + a_2b_2$). A tetrapolar mating pattern of quite the

same type has been observed by TAKEMARU (1957 c) at least in one case.

In rare cases the pairing pattern deviates from the normal tetrapolar scheme in that complete dicaryotization occurs in some few unexpected combinations (e.g. *8*, Fig. 3). Such deviations have occurred more frequently and regularly in part of TAKEMARU's material (1957 b, c). They may be connected with the compound character of certain incompatibility factors which allows occasional crossing over with the production of new "incompatibility factors" as a result (cf. *8*).

In pairing experiments the growth of the mycelia is sometimes changed in the contact zone which then appears as a distinct line of demarkation (see for example *2*, Photo 1). There were no indications of a connection between the occurrence of demarkation lines (or any type of such lines including "barrage" as described by VANDENDRIES & BRODIE 1933) and certain combinations of incompatibility factors of the meeting mycelia (*2*, p. 620). This result has been confirmed by the independent work of TAKEMARU (1954).

Some observations on the progress of the dicaryotization process between wildtype mycelia and mutant or "degenerate" mycelia have been reported in (*2*). A lasting state of unilateral dicaryotization (observations made after 18 days) was found in several combinations. It seems, for example, that in pairings between normal and degenerated mycelia only the former one is completely dicaryotized.

Studies on F_1-mycelia derived from combinations of normal and degenerate strains (*8*) showed the intercompatibility to be very low.

The genetical basis of fruit body formation (*2*, *8*).

During the studies on incompatibility factors it was observed that mycelia resulting from complete dicaryotization gave rise to normal fruit bodies, while dicaryotic mycelia from illegitimate pairings only formed fruit body primordia or incompletely developed fruit bodies (*2*). This observation should, however, not be generalized. L 1 × L 11 which is the result of an illegitimate mating ($a_1b_1 + a_2b_1$) formed normal fruit bodies during the first time after its synthesis (*4*, p. 388) though the capacity for such formation later disappeared. TAKEMARU (1957 b) has also described several cases of formation of perfectly developed fruit bodies from mycelia produced by illegitimate matings.

Occasional production of mature haploid fruit bodies has been observed for the strains F 34/110 (a cytidineless mutant of L 1) (*2*, p. 616) and L 7 (*7*, p. 291). The basidiospores from the latter showed low germination frequency (*8*, Table VIII). The capacity to form haploid fruit bodies was found again in the F_1 progeny from matings between F 34/110 and other strains and this capacity probably segregated independently from the character "cytidineless" (*2*).

Formation of haploid fruit bodies of *Collybia velutipes* has also been observed by other investigators (cf. *2*, p. 620; see also HELDMAIER 1929). Apparently the dicaryotic condition is not always necessary for the formation of fruit bodies, but it is certainly favourable for such formation, which may depend upon complementary action of the different nuclei and perhaps also of cytoplasmic factors.

Neohaplont variation (*1*, *8*).

As originally shown by HARDER (1927) for *Pholiota mutabilis*, neohaplonts obtained by microsurgical operations on dicaryotic mycelia sometimes show a wide range of variation, new types occurring in addition to the original ones used for the synthesis of the dicaryotic strain. Haplonts obtained in the same manner (one-cell isolations from monocaryotic mycelia by microsurgical technique) may be indistinguishable from each other as shown for *Polyporus abietinus* by FRIES & ASCHAN (1952). It is not easy to judge the extent of cytoplasmic mixing in the dicaryotization process (*1*, Fig. 1; FRIES & ASCHAN 1952, p. 441; PAPAZIAN 1958) but to some extent it probably takes place. A redistribution of stable cytoplasmic factors could then be the cause of the unexpected variation among the neohaplonts. Of course an uneven distribution of relatively unstable cytoplasmic factors generated during the dicaryotic stage would give a similar but transient result. Moreover, nuclei carrying mutated genes have better chances to accumulate in dicaryotic mycelia where they are supported by the other partner of the dicaryon, than in monocaryotic ones (cf. FRIES & ASCHAN 1952). To this may be added, finally, the probable occurrence of somatic recombination in the dicaryotic stage (cf. *8*, p. 325).

In *Collybia velutipes* the generation of monocaryotic oidia from dicaryotic mycelia offers another and more convenient possibility of obtaining neohaplonts which, like the operation neohaplonts, may show an extra variation. Oidial haplonts from the original mono-

caryotic mycelia have regularly been isolated for comparison, and have been found to be rather uniform in respect to morphology and biochemical behaviour. Some exceptions to this rule have, however, been observed. Oidial haplonts of two morphological types were thus obtained from a mycelium of F 36/198, which might be the result of spontaneous gene mutation. In the case of F 26/79 a variation similar to that of the oidial neohaplonts from A 1 × F 26/79 (cf. *8*, Table I and p. 313) occurred. It is conceivable that cytoplasmic factors might be unevenly distributed even during the growth and oidium formation of a monocaryotic mycelium, and this is a possible explanation of the fairly continuous variation of the oidial progeny of F 26/79. Some variation in, for example, growth rate does naturally occur among haplonts as well as neohaplonts (cf. *1*, p. 384–386).

All dicaryotic mycelia used for obtaining neohaplonts have been isolated from the contact zone of the monocaryotic mycelia used in their synthesis. The type of dicaryotization has been studied for many of the combinations (*2*, Table I) and it appears that deviating neohaplont types (*8*, Table I) have usually occurred from dicaryotic mycelia generated by incomplete (probable for F 29/157 × F 8/86) or unilateral dicaryotization. As far as known the only exception is the "regenerated wildtype" (a backmutation?) occurring among the neohaplonts from F 29/157 × F 36/257. It should be noted that all pairings involving F 31/40 (even F 31/40 × L 7, cf. *2*, Table I) have in later experiments remained unilaterally dicaryotized.

Takemaru (1957 a) has in one case compared the growth of two isolations of a mycelium generated by unilateral dicaryotization, one from the contact zone and the other as far from it as possible. No significant difference was found. Further experiments of this type, involving both incomplete, unilateral and bilateral dicaryotizations, are however desirable.

Several cases of deviating types among the oidial neohaplonts have been observed (*1*, *8*). To these a case of widely varying cellulolytic activity among neohaplonts may be added. L 1 shows a conspicuous cellulolytic activity while the morphological mutant F 36/242 (L 7) is almost devoid of such activity (Aschan & Norkrans 1953, p. 576). The oidial neohaplonts from L 1 × F 36/242 showed a wide range of variation in this respect, some types being even considerably more active than L 1 (unpublished).

The same alternatives of explaining the extra variation as discussed for the operation neohaplonts can naturally be applied to the oidial

neohaplonts. Under the circumstances, an exhaustive study of the F_1 progeny of strains giving rise to pronounced neohaplont variation was desirable. Unfortunately such a study has met with considerable difficulties, and it has not been possible to carry it to a conclusive stage within the compass of the present investigation. In some cases fruit bodies could not be obtained, or the basidiospores showed very low viability (*8*, p. 315). For some "degenerate" strains which dicaryotized normal mycelia unilaterally (on the side of the normal mycelium), preliminary studies on the F_1 progeny could, however, be made, though they were hampered by incomplete germination of the basidiospore samples and by depressed intercompatibility. The segregation of incompatibility factors gave, as mentioned above, indications of occasional crossing over within a "compound locus". The morphological characters showed a rather continuous variation which could be the result either of the cooperation of many different genes or of extrachromosomal factors. This continuous variation of the F_1 progeny was quite unexpected as preliminary experiments with L 1 and L 7 had given a quite clear segregation of the original morphological types.

Studies on a homothallic mycelium (*4*).

In a pairing between the two ornithineless mutants F 29/157 (L 1) and F 28/42 (L 11) a delayed clamp formation in the contact zone was observed (*4*). The clampforming mycelium (Mi 460) was isolated and found to grow well on a medium lacking ornithine. Quite unexpectedly, oidial isolations gave rise to clamp-forming mycelia only. Operation neohaplonts were unstable and soon reverted to clamp formation, a phenomenon which is characteristic for homothallic mycelia (HARDER 1926). The morphological differences between Mi 460 and a normal dicaryotic *Collybia velutipes* strain are rather pronounced, and this in connection with the homothallic behaviour of Mi 460 points to the possibility of classifying it as a new, artificially produced species. All efforts to obtain fruit bodies from Mi 460 were in vain.

Under the circumstances it became important to certify a true relationship between Mi 460 and normal *Collybia velutipes* strains, even if a contamination seemed hardly possible. Formation of anastomoses between Mi 460 and several other strains (F 28/42, F 29/157, L 1, L 11, L 7) were observed. Serological studies using precipitation

tests (including the gel diffusion test) showed the presence of common antigens in Mi 460, L 1 and F 28/42. One or more mutations, and perhaps also somatic recombination, must certainly be responsible for the genesis of Mi 460, which genesis shows that homothallism may not always be a primitive stage, but can also occur secondarily (cf. Olive 1958).

Physiology of fruit body formation (*3*, *5*, *6*, *7*).

As mentioned above the genetical analysis of certain interesting strains (including the homothallic Mi 460) was hampered by the failure of fruit body formation. It was therefore of interest to find the external conditions most favourable for such formation.

The positive effect of light, not on the initiation of primordia but on the normal development of the pileus, was well known. A study was, however, made on the influence of the quality of light on pileus growth (*6*). Only the blue end of the spectrum was active in causing such growth. Carotenoids were lacking in the fruit bodies and could thus not function as photoreceptors in this process.

The temperature optimum of the mycelial growth is in the range about 25°C for periods of growth shorter than about 35 days. For longer periods (where the growth of fruit body primordia may be of importance) the optimum tends to sink to the neighbourhood of 20°C (*7*). The production of fully developed fruit bodies in light, on the other hand, has its temperature optimum at +10° to +15°C, being earlier at the latter temperature but larger in the final state at 10°C; at 20°C the fruiting is rather inconstant and at 25°C no fruiting (not even primordia) has been obtained on the media preferentially used in the present investigation (*3*, *5*, *7*).

A pretreatment period of one week in darkness and at 25°C before placing the cultures in light gives a more profuse and uniform fruiting, even if its onset is often delayed for cultures on liquid media (*3*, *5*). This may be connected with a rapid accumulation of reserve materials during the pretreatment (cf. *5*, p. 326). Under no circumstances has the fruiting occurred after culture periods shorter than 3 weeks (including the pretreatment time).

The nutritional conditions have been varied within a rather wide range without any spectacular results in respect to earliness or profuseness of fruiting (*3*, *5*). Malt agar as well as two synthetic liquid media (*Min*: glucose, ammonium tartrate, thiamine and mineral

salts; *Pl*: sucrose, asparagine, thiamine and mineral salts) are roughly equivalent from this point of view. The carbon sources usually rate in the order: glucose > sucrose > maltose, and the nitrogen sources in the order: ammonium tartrate > ammonium chloride > asparagine > nitrate (not utilized). It should be mentioned that glucose media aquire a light yellow-brown colour during autoclaving and cases have been observed where unintentionally long autoclaving has resulted in increased mycelial growth (and depressed fruiting) (cf. 5, p. 320, also unpublished results).

Rising glucose concentration in the range 0.01 to 0.3 *M* had a clear depressing effect upon the rate of fruit body development; no compensating effect of increased thiamine concentration could be found. Increasing nitrogen supply often markedly depresses the fruiting of many fungi (cf. 5, p. 325; also Koch 1958). In the present case rising ammonium tartrate concentration had but a slight depressing effect and no clear interaction between the nitrogen and carbohydrate factors was apparent. Additions of acid hydrolysed casein, tryptophan and indoleacetic acid stimulated fruiting in some experiments, but the conditions necessary for a regular appearance of these effects cannot yet be specified. Though indoleacetic acid is known to occur in many fungi little or nothing is known with certainty about a growth regulating effect in these organisms (cf. Banbury 1959, Maas 1958, Gruen 1959). Some indications of the presence of a growth regulator of unknown nature in the gills have been adduced by Japanese workers (cf. Hagimoto & Konishi 1959).

Growth rate of mono- and di-caryotic mycelia (7).

As mentioned above nuclei carrying mutated genes must have better chances to maintain themselves in a dicaryotic mycelium, where possible deficiences are to some extent balanced by a complementary gene action, than in monocaryotic ones. From this point of view slowly growing monocaryotic mycelia should be expected to give rise to fast growing dicaryotic ones in some cases at least. Examples of this phenomenon are given in 7 (Table I). Many monocaryotic mycelia grow, however, as fast as dicaryotic ones. To the literature references mentioned in 7 may be added Kimura (1954), who showed that for three of the studied hymenomycetes especially the young dicaryotic mycelia grew much faster than monocaryotic ones. In two species the two types of mycelia grew at about the same rate.

Phosphorous and nitrogen metabolism (7).

The phosphorous (P) content of the studied *Collybia velutipes* mycelia varies from 1.3 to 3.7 % and the nitrogen (N) content from 1.7 to 10.5 % of the dry matter. The P content per unit dry weight tends to increase and the N content to decrease during the period of rapid growth. The P increase is located in the fraction soluble in 10 % trichloroacetic acid, while the P fraction comprising the nucleic acids instead decreases. Two compatible mycelia showed a consistent difference in the P content per unit dry weight, but further studies revealed that such differences may also occur between incompatible strains. External conditions favourable for fruit body production tend to lower the relative N and P content of the mycelia.

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